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Determination of Nitrogen by the Micro-Kjeldahl Method*

Previous studies (1–3) led to the establishment in 1950 of an official micro-Kjeldahl method which has given satisfactory results. Since that time, however, a better understanding of some of the factors affecting the method has been developed and modifications of the method have been made which appear to be an improvement, particularly in that digestion times were shortened markedly. One of the leaders in this work on the macro method was Perrin (4), who also proposed a simple method for testing the heat output of the digestion unit. This test has been adapted to digestion units for microanalysis and has been accepted as a part of the ASTM specification for micro-Kjeldahl apparatus.

With these new developments the possibility of improving the micro-Kjeldahl method seemed good, so a study was conducted this year to test a method designed to reduce the time required for digestion. At the same time it was hoped to improve interlaboratory precision by ensuring more reproducible digestion conditions through boiling-time specification for the heat output of the digestion units.

The present Kjeldahl method, **37.9**, is not applicable to samples that contain N-N or N-O linkages. Three attempts have been made (2, 3, 5) to find a suitable procedure for materials containing these linkages. In every case a number of collaborators obtained good results while others obtained low and erratic values. It was not possible to ascertain the cause for the differences because each collaborator felt that he had followed the written procedure faithfully. The obvious conclusion is that one or more steps or variables in the procedure are not defined sufficiently well, but it has not been

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possible to determine which these are. Steyermark (6) recently proposed a micro modification of Dickinson's Method for N-N and N-O-containing materials and it was decided to test this method collaboratively this year.

New Kjeldahl Method

Two pure samples, nicotinic acid and N (octadecyl) stearamide, were sent to collaborators with a copy of the micro-Kjeldahl procedure to be followed. The collaborators were asked to make quadruplicate analyses of both samples and to report all data obtained. Nicotinic acid was chosen because it is a refractory material and because it was used in previous studies, thus permitting a direct comparison of the data obtained this year with those obtained when the existing official procedure was established. The N (octadecyl) stearamide was selected to determine if the temperature could be increased enough during digestion to cause a loss of nitrogen. Its high carbon-to-nitrogen ratio would necessitate a larger than normal sample which would require more than the normal amount of sulfuric acid for its oxidation. This would raise the salt-to-acid ratio and, consequently, the boiling temperature of the digestion mixture.

Twenty-three analysts analyzed the two samples and obtained the results summarized in Table 1 where n is the number of determinations; $\bar{x}$, the mean of the analysts' values; s, the intralaboratory standard deviation; $\bar{\bar{x}}$, the mean of the $\bar{x}$ values; and s_m, the interlaboratory standard deviation. Only one collaborator failed to obtain reasonably good values for nicotinic acid. His values were very low and erratic and consequently were not included in the calculation of $\bar{x}$ and s_m values. The overall mean, $\bar{\bar{x}}$, was 11.34% as compared with the theoretical value of 11.38% and the value of 11.19% for the previous study (2). In the earlier study a number of individual values for several collaborators differed by more than 0.3% from the theoretical value, whereas in this study, all but two of the 87 values (excepting those of Collaborator 9) were within the ±0.3% range. Thus the intralaboratory precisions obtained with the new method were better than those for the present official method.

The interlaboratory precision was not calculated for the earlier study but inspection of the data shows that the s_m value would probably not have been as low as 0.100, the value for this study. The data for N (octadecyl) stearamide show that the method is equally suitable for this type of material. There is no evidence of nitrogen loss because of the larger sample weights required and consequent higher digestion temperatures. In fact the overall mean was 2.62% or 0.03% higher than theoretical. All but one of the intralaboratory standard deviations were 0.10 or less, averaging 0.04, and the interlaboratory s_m value was 0.081, a

Table 1. Results of collaborative test of new Kjeldahl procedure

Coll. No.	Sample 1 Nicotinic Acid (11.38% N)			Sample 2 N (Octadecyl) Stearamide (2.59% N)		
	n	$\bar{x}$	s	n	$\bar{x}$	s
0	4	11.37	0.02	4	2.59	0.01
2	4	11.49	0.16	4	2.65	0.04
8	4	11.41	0.18	4	2.57	0.06
9[a]	7	7.45	0.61	4	2.86	0.16
17	4	11.35	0.05	4	2.57	0.03
22	4	11.16	0.22	4	2.50	0.02
23	6	11.43	0.08	4	2.69	0.05
25	4	11.51	0.13	4	2.61	0.04
29	4	11.45	0.04	4	2.54	0.02
35	4	11.32	0.02	4	2.57	0.02
37	4	11.36	0.07	4	2.60	0.01
45	4	11.34	0.04	4	2.61	0.02
49	4	11.22	0.03	4	2.52	0.05
51	3	11.38	0.09	4	2.63	0.02
57	4	11.3	—	4	2.60	0.01
62	4	11.15	0.07	4	2.67	0.06
68	4	11.39	0.04	4	2.61	0.01
74A	3	11.22	0.13	4	2.62	0.10
B[b]	3	11.32	0.11	3	2.76	0.08
75	4	11.35	0.02	4	2.60	0.01
77	4	11.28	0.06	4	2.63	0.07
78	4	11.22	0.07	8	2.59	0.07
87	4	11.39	0.11	4	2.60	0.03
$\bar{\bar{x}}$		11.34	(0.03)		2.62	(0.04)
s_m		0.100			0.081	(0.04)

[a] Data from collaborator 9 not included in calculation of $\bar{\bar{x}}$ or s_m for sample 1.
[b] Analyst B used 1½ hour digestion for sample 1.

little lower than that for nicotinic acid. The slight difference between the s_m values for the two samples shows that with the method tested the agreement between laboratories is as good with refractory heterocyclic nitrogen compounds as with the less refractory amine or amide materials. Also the close agreement between $\bar{x}$'s and the theoretical values for both samples shows comparable accuracies for the two types of material.

The sum of the evidence indicates that the new Kjeldahl method tested this year is superior to the present official method not only in speed but also in accuracy and precision, and should replace the existing method.

METHOD

(NOT APPLICABLE TO MATERIAL CONTAINING N-N OR N-O LINKAGES)

Reagents

(a) *Sulfuric acid.*—Sp. gr. 1.84, N-free.

(b) *Mercuric oxide.*—N-free.

(c) *Potassium sulfate.*—N-free.

(d) *Sodium hydroxide-sodium thiosulfate solution.*—Dissolve 60 g NaOH and 5 g $Na_2S_2O_3 \cdot 5H_2O$ in H_2O and dilute to 100 ml or add 25 ml 25% $Na_2S_2O_3 \cdot 5H_2O$ to 100 ml 50% NaOH solution.

(e) *Boric acid solution.*—Saturated solution.

(f) *Indicator solution.*—(*1*) *Methyl red-methylene blue.*—Mix 2 parts 0.2% alcoholic Me red solution with 1 part 0.2% alcoholic methylene blue solution. Or (*2*) *Methyl red-bromocresol green solution.*—Mix 1 part 0.2% alcoholic Me red solution with 5 parts 0.2% alcoholic bromocresol green solution.

(g) *Hydrochloric acid.*—0.02*N*. Prepare as in **41.9** and standardize as in **41.13** or **41.15**.

Apparatus

(a) *Digestion rack.*—Use rack with either gas or electric heaters which will supply sufficient heat to 30 ml flask to cause 15 ml H_2O at 25° to come to rolling boil in not less than 2 or more than 3 minutes.

(b) *Distillation apparatus.*—Use one-piece or Parnas-Wagner distillation apparatus recommended by Committee on Microchemical Apparatus, ACS (7).

(c) *Digestion flasks.*—Use 30 ml regular Kjeldahl or Solty's type flasks.[1] For small samples, 10 ml Kjeldahl flasks may be used.

Determination

Weigh sample requiring 3–10 ml 0.01 or 0.02*N* HCl and transfer to 30 ml digestion flask. If sample weight is less than 10 mg, use micro balance. Weight should not be more than 100 mg dry organic matter. Use charging tube for dry solids, porcelain boat for sticky solids or non-volatile liquids and capillary or capsule for volatile liquids. Add 1.9 ± 0.1 g K_2SO_4, 40 ± 10 mg HgO, and 2.0 ± 0.1 ml H_2SO_4. If sample weight is more than 15 mg, add additional 0.1 ml H_2SO_4 for each 10 mg dry organic matter over 15 mg. Make certain that acid has sp. gr. of at least 1.84 if sample contains nitriles. (10 ml flasks and ½ quantities of reagents may be used for samples smaller than 7 mg). Add boiling chips which pass No. 10 sieve. If boiling time for digestion rack heaters is 2–2.5 minutes, digest 1 hour after all H_2O is distilled and acid comes to true boil; if boiling time is 2.5–3 minutes, digest 1.5 hours. (Digest 30 minutes if sample is known to contain no refractory ring *N*.)

Cool, add minimum quantity of H_2O to dissolve solids, cool, and place thin film of Vaseline on rim of flask. Transfer digest and boiling chips to distillation apparatus and rinse flask 5 or 6 times with 1–2 ml portions of H_2O. Place 125 ml Phillips beaker or erlenmeyer containing 5 ml saturated H_3BO_3 solution and 2–4 drops indicator under condenser with tip extending below surface of solution. Add 8–10 ml NaOH–$Na_2S_2O_3$ solution to still, collect about 15 ml distillate, and dilute to about 50 ml. (Use 2.5 ml H_3BO_3 and 1–2 drops indicator, and dilute to about 25 ml if 0.01*N* HCl is to be used.) Titrate to gray end point or first appearance of violet. Make blank determination and calculate % N = [(ml HCl − ml blank) × normality × 14.008 × 100]/mg sample.

Modified Kjeldahl Method

Eighteen analysts participated in the test of the method for materials containing N-N and N-O linkages. The data obtained are summarized in Table 2 where the symbols used are the same as those in Table 1. The results follow the same pattern shown in previous studies, i.e., some analysts obtained good results whereas others following the

[1] Available from apparatus supply companies.

Table 2. Results of collaborative test of Kjeldahl method modified for N-N and N-O containing materials

Coll. No.	Sample 3 Acetone-2,4-dinitrophenylhydrazone (23.52% N)			Sample 4 *p*-Nitrochlorobenzene (8.89% N)		
	n	$\bar{x}$	s	n	$\bar{x}$	s
0	4	23.18	0.29	4	8.81	0.01
2	4	22.80	0.23	4	8.61	0.04
9[a]	7	13.32	3.85	4	8.38	0.10
17	4	23.29	2.45	4	8.65	0.12
23	4	22.96	0.18	4	8.67	0.22
25[a]	4	18.31	3.60	4	8.07	0.06
29	4	23.70	0.11	4	9.04	0.07
35	4	22.64	0.13	4	8.58	0.06
37A[b]	4	22.94	0.17			
B	4	23.18	0.48	4	8.90	0.11
45	4	23.23	0.15	4	8.88	0.11
49	4	23.68	0.05	4	8.82	0.17
51	3	23.59	0.07	3	8.92	0.06
57	4	23.03	0.05	4	8.65	0.02
62	4	23.50	0.23	3	7.97	0.38
74	2	22.8	—	4	8.87	0.08
75	4	23.13	0.13	4	8.35	0.24
77	4	23.35	0.05	4	8.90	0.09
78	4	23.34	0.20	5	8.90	0.15
$\bar{x}$		23.20			8.67	
s_m		0.31			0.30	

[a] Data not included in calculation of $\bar{x}$ and s_m values for sample 3.
[b] A, 4 hours; and B, 2 hours digestion.

same procedure got low and erratic values. Only six analysts obtained what might be called acceptable means (23.52 ± 0.2%) for acetone-2,4-dinitrophenylhydrazone; the other 12 were all low although only two had values low by more than 1%. The results for *p*-nitrochlorobenzene were somewhat better with about half of the means and most of the standard deviations being acceptable. The interlaboratory means for both samples were low by about the same amount and the standard deviations high. Small differences in interpretation of the method details or small variation in conditions between laboratories apparently make the difference between good and poor results. Obviously the method will have to be refined before it can be suitable as an official procedure.

METHOD

(FOR MATERIALS WITH N-N AND N-O LINKAGES EXCEPT THOSE WITH N-N IN RING OR TRUE NITRATES)

Reagents

(a) to (g) See Kjeldahl method.

(h) Formic acid.—98–100% pure.

(i) Hydrochloric acid.—Sp. gr. 1.18, N-free.

(j) Zinc.—Powdered, N-free.

(k) Iron.—Powdered, N-free, prepared by hydrogenation.

(l) Ethyl alcohol.—95%.

Apparatus

Same as for Kjeldahl method plus water bath at 80–85°C.

Determination

Weigh 5–10 mg sample, transfer to a 30 ml Kjeldahl flask (preferably a Solty's flask), add 0.2 ml formic acid and 0.1 ml HCl, and heat in water bath at 80–85°C until sample is dissolved. Add 80 mg Zn, swirl for about 2 minutes to mix, and place in a water bath for 5 minutes. Add 40 mg Fe, swirl for 2 minutes, add 0.1 ml HCl and 0.15 ml ethyl alcohol, and heat 5 minutes in water bath. Leave flask in bath and add 0.1 ml portions of HCl every 5 minutes until Fe is dissolved. Add 1 ml H_2SO_4 while swirling flask in hood and warm until HCl is expelled. Add 0.65 g K_2SO_4, 16 mg HgO, and an additional 0.5 ml H_2SO_4. Digest 4 hours (if sample contains no refractory ring nitrogen, digest 1 hour after acid boils) and complete determination as under Kjeldahl method.

For sample weights between 10 and 20 mg, double the amount of all reagents added.

Recommendations

It is recommended[1]—

(1) That the micro-Kjeldahl method tested this year be adopted as first action.

(2) That the existing micro-Kjeldahl method **37.9–37.11** be deleted.

(3) That the study of Kjeldahl methods modified to determine nitrogen in materials containing N-N and N-O linkages be continued.

[1] These recommendations were approved by Subcommittee C and were adopted by the Association. See *This Journal*, **43**, 133 (1960).

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